Urinary exosome-derived microRNAs as biomarkers of tenofovir disoproxil fumarate-associated (TDF) renal toxicity in people living with HIV-1

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BACKGROUND

The incidence, severity, and outcome of TDF-associated renal toxicity is yet controversial and therefore the use of sensitive biomarkers for renal toxicity and risk of renal deterioration/CKD diagnosis is still needed.

MicroRNAs (miRNAs) are endogenously produced, short RNAs of 21–25 nucleotides that do not code for proteins, but regulate their expression levels via post-transcriptional. In kidneys, miRNAs are indispensable for its development and homeostasis.

The aim of this study was to evaluate the expression profiles of urinary exosome-derived microRNAs (miR) as biomarkers of TDF-associated renal toxicity and its usefulness in identifying and predicting tubular and renal involvement as manifestation of toxicity for TDF

METHODS

This study was the continuation of a longitudinal observational study about TDF associated renal toxicity (NCT02209740). Excluded individuals: pregnant or lactating women and individuals receiving investigational, nephrotoxic, or other drugs known to impair the function of the multidrug resistant protein 4 (MRP4) involved in the active secretion of TDF in the tubular cells.

Urine samples of 60 random virologically suppressed PWH who had at least one year of exposure of TDF-based antiretroviral therapy.

Renal tubular dysfunction (TD) was defined as the presence of at least two tubular abnormalities known to be part of Fanconi's syndrome (nondiabetic glycosuria, hyperuricosuria, and/or proteinuria).

A battery of 8 pre-defined miR were finally evaluated. These miR were selected from published literature about miR and renal evaluation.

Urinary exosomes isolation: exosomes were precipitated using the miRCURY Exosome isolation kit (Exiqon A/S, Vedbaek, Denmark) and analysed to control the purity and yield by quantification for exosome content by the ExoELISA-ULTRA CD63 assay (SBI System Bioscience, Mountain View, CA, USA). RNA was extracted from isolated exosome-enriched pellets using the miRCURY RNA isolation kit-Biofluids (Exiqon A/S).

Statistical analysis: two-sided test was used for all statistics and p<0.05 was considered statistically significant. Data analysis was performed using Statistical Package for the Social Sciences Software (SPSS Version 22.0, Chicago, Illinois)

This is the first study showing the usefulness of micro-RNA as biomarkers of antiretroviral drug-associated toxicity.

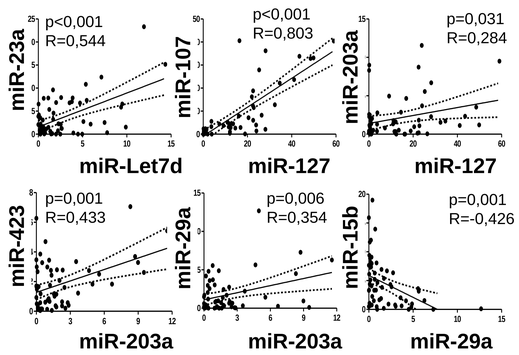
The expression profile of miRs was significantly altered in urinary exosomes according to time on TDF, changes in tubular parameters and presence of tubular dysfunction, and were associated with further tubular dysfunction and eGFR decrease.

Exosome-derived miRs in urine could be used as non-invasive biomarkers for the detection of renal toxicity associated with TDF.

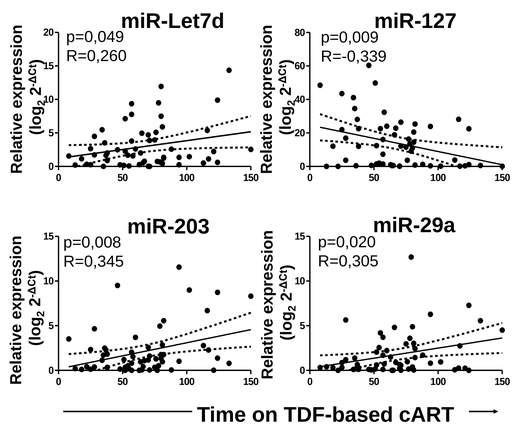
RESULTS

Clinical variables	Median (IQ_{25-75})
Mean age (years, range)	54 (33-84)
Sex male at birth	50 (83)
Risk practice for HIV infection	
Former IVDU	19 (32)
MSM	29 (48)
BMI (kg/m2)	23.6 (21-28.9)
Nadir CD4+ count (cells/mcL)	212 (119-301)
Time of HIV infection (months)	118 (57-149)
cART at syphilis diagnosis	60 (100)
cART including cobicistat/ritonavir	11 (18)
CD4+ count (cells/mcl)	553 (348-745)
HIV RNA level <50 cop/ml	60 (100)
Time on TDF (months)	65 (38-82.9)
Serum analysis	
eGFR-screa (CKD-epi)	90.9 (78-103.3)
<60 ml/min/1.73m2	4 (6)
eGFR-cysC	89.4 (76-104.3)
<60 ml/min/1.73 m2	2 (3)
Serum phosphate (mg/dl)	2.9 (2.4-3.5)
25-OH-vitamin D (ng/ml)	18.8 (12.7-28-9)
PTH (pg/ml)	55 (48-68)
Urine determinations	
Protein/creatinine ratio (mg/g)	99.3 (77-127.3)
Albumin/creatinine ratio (mg/g)	6.9 (4.4-12.2)
FE of phosphate (%)	24 (17-28.8)
%≥ 20%	37 (62)
Glycosuria (≥100 mg/dl)	7 (12)
RBP (mcg/g)	458 (350-891)
B2M (mcg/g)	5267 (2667-16678)
Tubular dysfunction (≥2 abnormalities)	25 (42)

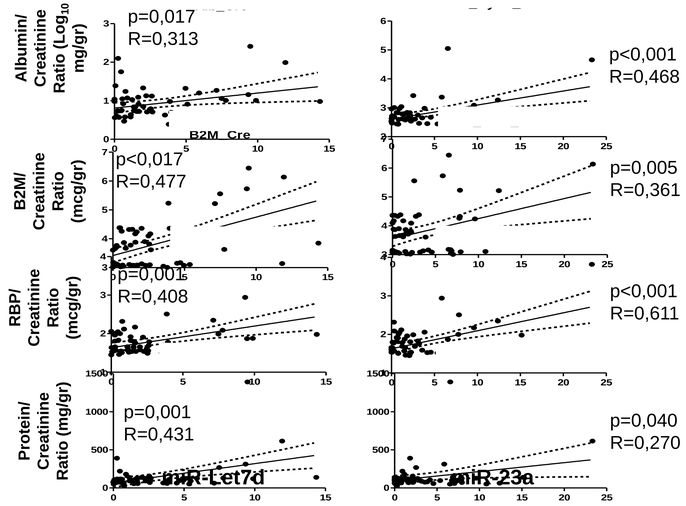
Correlations among relative expression levels (Log_2 $2^{-\Delta Ct}$) **of miRs** of the 60 HIV-1-infected individual



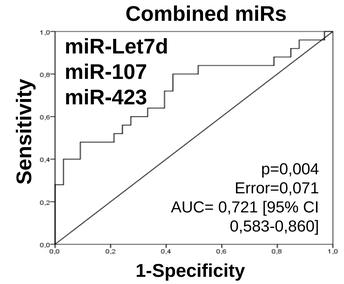
Correlations of miRs with the time of exposure to TDF-based ART in the 60 HIV-1-infected individuals included in the study



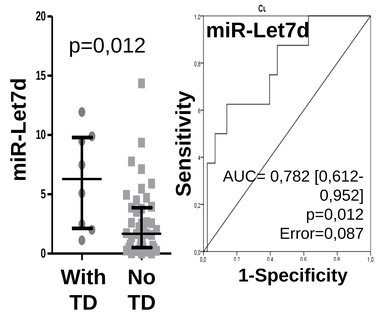
Correlations between **relative expression levels of miRs** (Log_2 $2^{-\Delta Ct}$) and the different tubular parameters



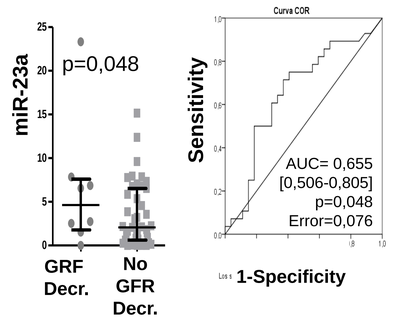
ROC curve showing the **fraction of true positive results** (sensitivity) **and false positive results** (1-specificity) for levels of the combination of miR-Let7d, miR-107 and miR-423 as diagnostic biomarkers of TD.



Differences in the relative expression levels of **miR-Let7d** (log2 2-ΔCt) between individuals with **tubular dysfunction (TD)** and with no TD after a median time of follow up of 9 months.



Differences in the relative expression levels of **miR-23a** (log2 2-ΔCt) between individuals who had **eGFR-cysC decrease** and individuals who had not after a median time of follow up of 9 months.



ADDITIONAL KEY INFORMATION

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